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(57) Abstract

10-Oxo-11,33-dihydropenitrem B has been isolated from the sclerotia of the fungi *Aspergillus sulphureus*, and 14-hydroxypaspalinine and 14-(N,N-dimethylvalyloxy)paspalinine have been isolated from the sclerotia of the fungi *Aspergillus nomius*. The indole compounds are effective for controlling Coleopteran and Lepidopteran insects.

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INDOLE ANTIINSECTAN METABOLITES

Background of the Invention

Field of the Invention

The present invention is generally related to indole compounds. More specifically, the indole compounds are used as insecticides for control of Lepidoptera and Coleoptera species.

Background of the Art

Certain fungi produce specialized resting bodies known as sclerotia as a means for surviving adverse environmental conditions which other fungal bodies cannot tolerate, such as harsh climate, nutrient deficiency and desiccation. Generally, sclerotia remain viable in soil for periods of several years, and provide primary inoculum for the producing species when conditions again become favorable for fungal growth. Sclerotia are formed under natural conditions or in solid substrate fermentations, but are not commonly produced in the liquid fermentation cultures generally employed in studies of microbial metabolites. Accordingly, many novel sclerotial metabolites of common fungi such as *Aspergillus* have not been characterized.

While sclerotia are known to contain biologically active secondary metabolites not found in other fungal parts or in liquid cultures, study of sclerotia as sources of novel metabolites has been limited. Investigation of large sclerotia (ergots) of *Claviceps purpurea* led to the discovery and medicinal use of ergot alkaloids.

Sclerotia have recently been recognized as a valuable potential source for natural antiinsectans. Many sclerotia, which are subjected to predation by fungivorous insects and arthropods during their period of dormancy in soil, have been shown to contain metabolites that exert adverse physiological effects on insects. Gloer et al. [*J. Org. Chem.* 53:5457 (1988)] and Wicklow et al. [*Trans. Br. Mycol. Soc.* 91:433 (1988)] disclose the isolation of four antiinsectan aflavanine derivatives from the sclerotia of *Aspergillus flavus* for use in controlling the dried-fruit beetle *Carpophilus hemipterus*

(Nitidulidae:Coleoptera). TePaske et al. [*J. Org. Chem.* 55:5299 (1990)] disclose a related metabolite, aflavazole, which was isolated from extracts of *A. flavus* sclerotia. Gloer et al. [*J. Org. Chem.* 54:2530 (1989)] describe an insecticidal indole diterpene known as nominine found only in the sclerotia of *Aspergillus nomius* for the control of the corn earworm *Helicoperva zea* (Lepidoptera), formerly *Heliothis zea*. Nominine is also disclosed by Dowd et al. in U.S. Patent No. 5,017,598 issued May 21, 1991, and entitled "Nominine, an Insecticidal Fungal Metabolite".

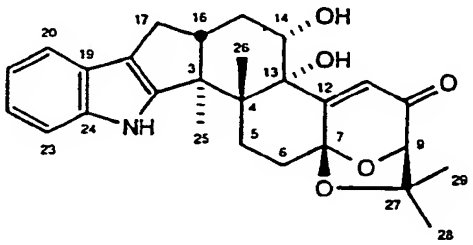
The compounds penitrem A-F [de Jesus et al., *J. Chem. Perkin Trans. I*, 1847-1861 (1983)] and aflatrem [Gallagher et al., *Tetrahedron Lett.* 21:239 (1980)] are known tremorgenic mycotoxins which are produced from strains of *Penicillium crustosum* and *Aspergillus flavus*, respectively. Paspalinine, paspalicine and paspaline from *Claviceps paspali* are also known to cause tremors in mice and domestic animals [Gallagher et al., *Tetrahedron Lett.* 21:235 (1980); Springer and Clardy, *Tetrahedron Lett.* 21:231 (1980)]. A mechanism of action for these tremorgens is proposed by Setala et al., *Drug Chem. Toxicol.* 12:237 (1989).

Tremorgenic mycotoxins such as penitrem A, aflatrem and paspaline are described by Dowd et al. as possessing insecticidal activity [U.S. Patent No. 4,973,601, issued November 27, 1990; *J. Antibiot.* 41:1868 (1988)] Dowd et al. disclose a method of controlling insects such as *H. zea* and *S. frugiperda* by applying a fungal tremorgenic metabolite containing an indole moiety to a locus of insects.

There remains a continuing need for new insecticides because many agriculturally important insect species have developed a resistance to the most potent insecticides which are currently available. Moreover, environmentally tolerable replacements for these insecticides are declining. New natural, biodegradable insecticides which are relatively nontoxic to vertebrates and may be produced by fermentation processes are a cost effective replacement for known insecticides.

Summary of the Invention

In order to satisfy the need for a cost effective, natural, biodegradable insecticide, one aspect of the present invention provides substantially pure indole compounds which are effective for controlling Lepidopteran and Coleopteran insects. 14-Hydroxypaspalinine has the formula:



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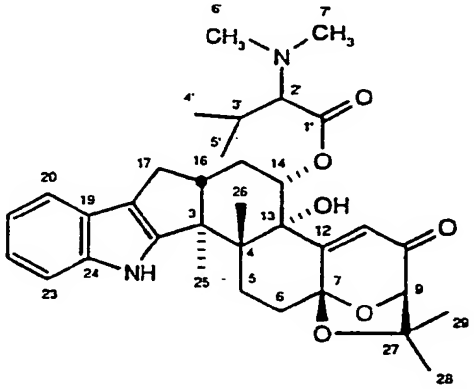
14-(N,N-dimethylvalyloxy)paspalinine has the formula:



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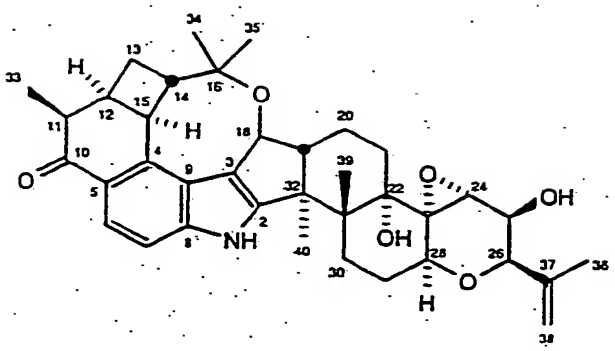
and 10-oxo-11,33-dihydropenitrem B has the formula:



Another aspect of the present invention provides a composition for controlling insects containing a 14-hydroxypaspalinine, 14-(N,N-dimethylvalyloxy)paspalinine, or 10-oxo-11,33-dihydropenitrem B compound and an inert carrier. The compound is preferably present in the composition in an amount effecting insects of the Lepidopteran or Coleopteran species, such as *Helicoverpa zea* or *Carpophilus hemipterus*. An effective amount of the composition may be applied to a locus of insects in order to control the insects.

Detailed Description of the Invention

The present invention provides several substantially pure indole compounds effective in controlling insects, insecticidal compositions containing a compound of the present invention and a method for controlling insects by applying the compositions to the locus of the insects. The compounds of the present invention have been designated 14-hydroxypaspalinine, 14-(N,N-dimethylvalyloxy)paspalinine, and 10-oxo-11,33-dihydropenitrem B, and are collectively referred to as "the compounds."

10-Oxo-11,33-dihydropenitrem B is isolated from the sclerotia of the fungus *Aspergillus sulphureus*, a member of the *A. ochraceus* taxonomic group. 14-Hydroxypaspalinine and 14-(N,N-dimethylvalyloxy)paspalinine

are isolated from the sclerotia of the fungus *Aspergillus nomius*. Strains of the fungi *Aspergillus sulphureus* and *Aspergillus nomius* were deposited on June 11, 1991, June 10, 1991, and December 19, 1989 respectively, in the Agricultural Research Service Patent Culture Collection (NRRL) in Peoria, IL and have been assigned respective Deposit Nos. NRRL 18838 and 18585. The culture deposit will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms. All restrictions on the availability of the culture deposit to the public will be irrevocably removed upon the granting of a patent disclosing the strain.

The sclerotia of *A. sulphureus* and *A. nomius* are produced by solid-substrate fermentation on corn kernels. They are ground by conventional means to a suitable particle size and are extracted with at least one solvent. Suitable solvents for the extraction could be readily determined by the skilled artisan and would include any solvents in which the compounds of the present invention are soluble. Preferably, the ground sclerotia of *A. sulphureus* are sequentially extracted with pentane and methylene chloride. *A. nomius* sclerotia are preferably extracted with pentane to extract 14-(N,N-dimethylvalyloxy)paspalinine, and are sequentially extracted with hexane and chloroform to extract 14-hydroxypaspalinine.

Isolation and purification of the compounds of the present invention from the solvent extract is effected by the use of conventional techniques, such as high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), silica gel column chromatography and countercurrent distribution (CCD). In a preferred embodiment of the invention, a solvent extract is separated by silica gel column chromatography, and the resulting fraction is further separated by reversed-phase HPLC. 10-Oxo-11,33-dihydropenitrem B is isolated using this procedure as described in Example 1, although the procedure is not limited thereto. In another preferred embodiment described in Example 3, solvent

extracts are subjected to reversed-phase HPLC to yield 14-hydroxypaspalinine and 14-(N,N-dimethylvalyloxy)paspalinine.

Commercial formulations including the compounds of the present invention may be prepared directly from fungal extracts or from the fractions derived from the extracts. However, the formulations are prepared from a pure or a substantially pure compound when a high degree of specificity is required. For example, if a high degree of predictability of the intended response by both target and nontarget species is required, a formulation prepared from a pure or substantially pure form of a compound of the present invention would be used. The formulation would then exclude other substances found in natural fungi which might have an adverse effect on activity or a toxic effect toward nontarget species.

Insecticidal compositions of the present invention include 14-hydroxypaspalinine, 14-(N,N-dimethylvalyloxy)paspalinine, or 10-oxo-11,33-dihydropenitrem B as described above in combination with a suitable inert carrier as known in the art. Agronomically acceptable carriers such as alcohols, ketones, esters and surfactants are illustrative. A compound of the present invention is present in the composition in an amount effecting the target species which is typically at least about 1.0 ppm. The concentration of the compound in an insecticidal composition will vary considerably depending upon the target species, substrate, method of application and desired response. Addition factors to be considered in determining an optimum concentration include phytotoxicity toward the treated plant and the tolerance of nontarget species.

The compounds of the present invention act to control pests by mechanisms including growth regulation, death inducement, sterilization, as well as interference with metamorphosis and other morphogenic functions. The resulting response is dependant on the pest species, the compound concentration and method of application. The compound is administered in an amount effecting one or more of the responses as may be predetermined by routine testing. Where the intended response is pest mortality, an

"effective amount" is defined as the quantity of the compound which will effect a significant mortality rate of a test group as compared with an untreated group. The actual effective amount will vary with the species of pest, stage of larval development, nature of the substrate, the type of inert carrier, the period of treatment and other related factors.

The compositions of the present invention are effective in controlling a variety of insects. Agronomically important insects such as those of the orders Lepidoptera and Coleoptera are of particular interest. However, the compounds and compositions of the present invention are not limited thereto.

The insecticidal compositions of the present invention are used to control insects by applying the composition to the locus of the pest to be controlled. When the compound is intended as a stomach poison, it is applied in conjunction with an inert carrier to the pest diet. The composition is applied to plants by treating the leaf surfaces or by systematic incorporation. As a contact poison, any topical method of application will be effective, such as direct spraying on the pest or on a substrate which is likely to be contacted by the pest.

The following examples are presented to describe preferred embodiments and utilities of the present invention and are not meant to limit the present invention unless otherwise stated in the claims appended hereto.

Example 1: Isolation and Purification of 10-Oxo-11,33-dihydropenitrem B

The culture of *A. sulphureus* (NRRL 18838) was obtained from the Agricultural Research Service (ARS) culture collection at the National Center for Agricultural Utilization Research in Peoria, IL. Production of sclerotia was accomplished by solid substrate fermentation of *A. sulphureus* on autoclaved corn kernels using procedures described by Wicklow et al., *supra* (1988). The sclerotia were harvested, ground to a powder using a Tecator mill obtained from Perstorp Instrument Co. and stored at 4°C until extraction.

Powdered sclerotia of *A. sulphureus* (150.0 g) were sequentially extracted with pentane and methylene chloride using a Soxhlet apparatus. A portion (894 mg) of the total methylene chloride extract (1.59 g) was fractionated by silica gel column chromatography. A stepwise gradient from 0-10% (v/v) methanol in chloroform was employed resulting in the elution and collection of a distinct red band at 4% methanol. The fraction that yielded 10-oxo-11,33-dihydropenitrem B eluted immediately after this distinct band. This active fraction (50.1 mg) was further separated by reversed-phase HPLC (92:8 MeOH-H_2O) to yield 9.7 mg 10-oxo-11,33-dihydropenitrem B as a light yellow solid.

10-Oxo-11,33-dihydropenitrem B has the following properties: HPLC retention time 8.9 min; $[\alpha]_D$ -78.6° (c = 0.002 g/ml); ^1H NMR, ^{13}C NMR, HMBC, and NOESY data in Table 1; EIMS (70 eV): *m/z* 599 (M^+; rel. int. 26), 493 (15), 469 (26), 400 (9), 265 (12), 264 (65), 134 (26), 133 (23), 131 (23), 130 (100), 119 (54); HREIMS: obsd. 599.3244; calcd. for $C_{37}H_{45}NO_6$, 599.3247.

In determining the properties of the compounds, proton NMR and heteronuclear multiple bond correlation (HMBC) data were obtained on a Bruker AMX-600 spectrometer. ^{13}C NMR data was obtained using Bruker AC-300 or WM-360 spectrometers. Heteronuclear multiple quantum correlation (HMQC) experiments were conducted using MSL-300 or AMX-600 spectrometers. All spectra were recorded in $CDCl_3$, acetone-d_6, or CD_3OD, and chemical shifts were referenced using the corresponding solvent signals: 7.24 ppm/77.0 ppm, 2.04 ppm/29.8 ppm, or 3.30 ppm/49.0 ppm, respectively. Multiplicities of carbon signals were determined through distortionless enhancement by polarization transfer (DEPT) experiments. Selective insensitive nuclei enhanced by polarization transfer (INEPT) experiments were optimized for $^nJ_{CH}$ values of 4, 7 or 10 Hz, while the HMBC experiments were optimized for $^nJ_{CH}$ = 8.3 Hz. A VG TRIO-1 mass spectrometer equipped with a direct inlet probe was used to obtain El mass spectra at 70 eV. High resolution electron impact mass spectrometry

(HREIMS) data were acquired with a VG ZAB-HF instrument. HPLC separations were accomplished using a Beckman Ultrasphere ODS column (5-μm particles, 250 x 10 mm) at a flow rate of 2.5 ml/min; UV detection was at 215 nm. Penitrem B has an HPLC retention time under the above conditions of 20.1 min.

Table 1. Spectral Data For 10-Oxo-11,33-dihydropenitrem B[a]

C/H#	^{1}H	^{13}C	HMBC Correlations	NOESY Correlations[c]
2	—	155.1		
3	—	120.8		
4	—	136.5		
5	—	125.8		
6	7.38 (d; 8.5)	118.9	4,7, 8, 9[b], 10	
3	7.18 (d; 8.5)	111.4	4[b], 5, 9	
8	—	143.4		
9	—	122.7		
10	—	203.8		
11	2.81(m)	46.6	10, 12, 13, 33	
12	2.27(m)	34.4	13, 14, 33	33
13a	2.18	28.7	11, 12, 14, 16	
13b	1.91(m)	—	11, 12, 15	
14	2.77(m)	50.5	4, 13, 15, 16, 34, 35	35
15	3.83(dd; 9.2, 9.2)	35.7	4, 5, 9, 11, 12, 14, 16	18, 34
16	—	77.3		
18	4.89(d; 8.2)	73.2	2, 3, 16, 19, 20	15, 34, 40
19	2.65(m)	59.9	18, 20, 21, 32, 40	39
20ax	1.93(m)	18.8	19, 21, 32	40
20eq	1.77(m)	—	19, 22	
21ax	1.47 (m)	30.5	19	24
21eq	1.74(m)	—	19, 22	
22	—	78.5		
23	—	66.7		

24	3.49 (br s)	62.2	25, 26	21ax, 36, 39
25	4.04(br s)	66.5	28, 36, 37, 38	28
26	4.03 (br s)	75.1	23, 24, 26	
28	4.29 (dd; 9.2, 8.6)	72.7	23, 24, 29	25
29ax	2.10(m)	29.0	28, 30	39
29eq	2.29(m)	—	23, 28, 30, 31	
30ax	2.61(m)	27.0	22, 29, 31, 39	40
30eq	1.62(m)	—	22, 28, 29, 31, 32	
31	—	44.0		
32	—	50.9		
33	1.14(d; 6.4)	12.7	10, 11, 12	12
34	1.55(s)	19.0	14, 16, 35	15, 18, 35
35	1.20(s)	28.7	14, 16, 34	14, 34
36	1.71(br s)	19.8	26, 37, 38	24, 38b
37	—	143.2		
38a	5.09 (br s)	111.9	26, 36, 37	
38b	4.91 (br s)	—	26, 36	36
39	1.23(s)	19.0	22, 30, 31, 32	19, 24, 29ax
40	1.42(s)	21.2	2, 19, 31, 32	18, 20ax, 30 ax

[a]Data were recorded in CD_3OD at 600 and 75.6 MHz, respectively.
[b]These correlations represent 4-bond couplings; all other HMBC correlations represent 2-or 3-bond couplings.
[c]NOESY correlations between scalar coupled protons have been omitted.

The molecular formula of 10-oxo-11,33-dihydropenitrem B was determined to be $C_{37}H_{45}NO_6$ by analysis of HREIMS data. This formula is identical to that of penitrem E [de Jesus, et al. *supra* (1983)]. Penitrem E is hydroxylated at C-15, but otherwise identical to penitrem B. Comparison of the ^{13}C NMR data for penitrems B and E with those of 10-oxo-11,33-dihydropenitrem B indicated that one methylene and two vinylic carbon signals in the ^{13}C NMR spectrum of penitrem B appear to be replaced with new methyl, methine, and ketone carbon signals in the ^{13}C NMR spectrum of 10-oxo-11,33-dihydropenitrem B.

^{13}C and ^{1}H NMR assignments for 10-oxo-11,33-dihydropenitrem B are provided in Table 1. Carbon-proton one-bond correlations were made by analysis of an HMQC spectrum. By comparing HMBC data obtained for 10-oxo-11,33-dihydropenitrem B with the data published for secopenitrem B and penitrem B, it was determined that the indole nucleus as well as the entire right-hand portion of the molecule (rings F-I) were intact. The location of the ketone carbonyl functionality at C-10 was established on the basis of an HMBC correlation (Table 1) between H-6 and the ketone carbon (C-10). The methyl doublet (for H_3-33) also shows a correlation to the ketone carbonyl signal C-10, demonstrating that the methine is directly connected to C-10. In addition, the corresponding methine proton (H-11) shows correlations to C-10, C-12, C-13, and C-33, thereby confirming the location of the CH-CH_3 unit. HMBC data indicated that the remainder of the molecule is identical to the corresponding portion of penitrem B.

The relative stereochemistry of 10-oxo-11,33-dihydropenitrem B was deduced by examination of nuclear overhauser enhancement/exchange spectroscopy (NOESY) data and by analogy to penitrem B. Axial and equatorial proton dispositions were assigned based on coupling constants, NOESY correlations, and comparisons to the data for penitrem B. All NOESY correlations are consistent with the relative stereochemistry proposed for penitrem B [de Jesus et al. *supra* (1983)]. The assignment of the new methyl group (CH_3-33) to an equatorial position was based on its NOESY correlation to H-12. This correlation would be unlikely if CH_3-33 were in an axial position.

Example 2: Insecticidal Activity of 10-Oxo-11,33-dihydropenitrem B

The compound was evaluated by insect bioassays described previously by Dowd in *Entomol. Exp. Appl.* 47:69 (1988). Neonate larvae of *H. zea*, second instar (*ca.* 0.75 mg) larvae of *C. hemipterus* and adults of *C. hemipterus* were used for all assays. They were obtained from laboratory

colonies reared on a pinto bean-based diet at 27°C ± 1°C, 40 ± 10% relative humidity, and a 14:10 light:dark photoperiod.

The diet used to rear the insects was based on a standard pinto bean diet for many species, which contains the following ingredients: 120 g dried pinto beans, 43 g wheat germ, 28 g brewer's yeast, 8 g Vanderzant's vitamin mix, 2.8 g ascorbic acid, 1.75 g methyl paraben, 0.9 g sorbic acid, 12 g agar, 2 ml formaldehyde (38%), 1.5 ml of propionic-phosphoric acid solution (42% propionic acid, 4.2% phosphoric acid), and 550 ml water. All dry diet ingredients (except for the pinto beans) were purchased from U.S. Biochemicals Corp. Before use, the beans were soaked in water until saturated (overnight). The agar was added to 250 ml of water and brought to a boil. The other ingredients were blended in a Waring blender until uniformly mixed. The hot agar was added, and blending continued until all ingredients were uniformly mixed.

The pinto bean-based diet thus prepared was added in 5-ml quantities to test tubes. The test tubes were held at 60°C until chemicals were incorporated to prevent solidification of the diet. A compound of the present invention was added in 125 μl of acetone to the liquid diet to give a final concentration of 100 ppm. Upon addition of the compound, the mixture was removed from the water bath. The chemical was incorporated into the diets by blending vigorously with a vortex mixer for 20 sec. Preliminary observations with colored solutions of both water and acetone indicated uniform incorporation by this method. The diets were dispensed into culture plates and allowed to cool to room temperature. To remove the potentially toxic acetone, the diets were placed in a fume hood for *ca.* 20 min until slight darkening occurred. The diets were cut into approximately equal sections, and each section was placed into a well of a 24-well immunoassay plate. A single neonate *H. zea* or five *C. hemipterus* larvae or adults was added to each well. To prevent desiccation of the diet, the plate was covered by a sheet of parafilm, a sheet of cardboard, and the plastic cover. The cover was secured by two rubber bands, and groups of plates were

placed in two polyethylene bags held closely by rubber bands. The plates were held under the same conditions used to rear the insects. Mortality was checked at 2, 4 and 7 days, and the surviving larvae were weighed after 7 days. Each chemical set was tested on a total of 20 larvae. Diet feeding rating for *C. hemipterus* larvae was based on a scale of 1 (limited to no feeding) to 4 (diet thoroughly tunneled or pulverized) [Wicklow et al., *supra* (1988)].

10-Oxo-11,33-dihydropenitrem B exhibits antiinsectan activity against *Helicoverpa zea* and *Carpophilus hemipterus*. A 95.1% reduction in weight gain relative to controls after one week was noted for the 10-oxo-11,33-dihydropenitrem B when incorporated into a standard *H. zea* diet at 100 ppm. A feeding reduction of 33% was induced in the adults of *C. hemipterus*.

Example 3: Isolation and Purification of 14-Hydroxypaspalinine and 14-(N,N-Dimethylvalyloxy)paspalinine

Fungal isolates of *A. nomius* (NRRL 18585) were obtained from the ARS collection at the USDA Center for Agricultural Utilization Research in Peoria, IL. The sclerotia were prepared by solid substrate fermentation on autoclaved corn kernels using procedures described by Wicklow et al. *supra* (1988), and were stored at 4°C until extracted.

Ground *A. nomius* sclerotia (16.3 g) were extracted with hexane, followed by chloroform (5 x 50 ml each). Evaporation of the solvent from the combined chloroform extracts afforded 59.3 mg of a yellow oil. A portion of the extract (49.6 mg) was subjected to reversed-phase semipreparative HPLC (8:2 MeOH:H_2O at 2.0 ml/min) to afford 1.6 mg of 14-hydroxypaspalinine having the properties: ^1H NMR, ^{13}C NMR, HMBC, and NOESY data, Table 2; EIMS (70eV): *m/z*449 (M^+; rel. int. 3%), 434 (4), 358 (1), 285 (0.5), 265 (0.6), 212 (2), 182 (10), 168 (89), 130 (9), 100 (14), 44 (65), 43 (26); HREIMS: obsd. 449.2185; calcd. for $C_{27}H_{31}NO_5$, 449.2202.

A. nomius sclerotia (58.9 mg) was Sohxlet-extracted with pentane for 6 days. Filtration and evaporation of the solvent afforded 377.6 mg of a light yellow oil. This residue was subjected to reversed-phase preparative HPLC (85:15 MeOH:H_2O at 11.2 ml/min), to obtain 39.6 mg of 14-(N,N-dimethylvalyloxy) paspalinine with the following properties: ^1H NMR, ^{13}C NMR, HMBC data, Table 3; UV (MeOH) λ_{max}228 (ε15620), 279(3110); IR (neat) 3399, 2936, 1734, 1690 cm^{-1}; FABMS (3-NBA matrix) *m/z* 577 [$(M + H)^+$, rel. int. 100%], 576 (40), 575 (18), 533 (3.9), 519 (5.2), 449 (2.1), 448 (4.2), 431 (3.8), 390 (4.4), 374 (6.7), 358 (5.2); HRFABMS: obsd. 577.3278; calcd. for $C_{34}H_{45}N_2O_6(M + H)^+$, 577.3276.

In determining the properties of the compounds, carbon multiplicities were determined by DEPT experiments, and are consistent with the assignments. 2D-NMR experiments were conducted at 600 MHz (^1H dimension). HMBC and HMQC experiments were optimized for $^nJ_{CH}$ values of 8.5 and 135 Hz, respectively. Selective INEPT experiments were optimized for $^nJ_{CH}$=7Hz. Reversed-phase preparative HPLC was accomplished using a Rainin Dynamax-60A 8μ C_{18} column (21.4mm x 25 cm).

Table 2. NMR Data For 14-Hydroxypaspalinine in $CDCl_3$

Position	^1H	^{13}C	HMBC Correlations	NOESY Correlations[a]
1	7.69(s)	–	2,18,19,24	5,23
2	–	151.0	–	
3	–	50.5	–	
4	–	40.1	–	
5ax 5eq	2.68(dd, 12.5, 10.4) 1.79(ddd,12.7,10.0,8.9)	27.6	3,4,6,7 4,6,13	25 26
6ax 6eq	2.84(m) 2.00(m)	28.5	4,5,7 4,7,12	26
7	–	104.7	–	
9	4.30(br s)	88.1	7,10,11,28	28,29
10	–	197.8	–	
11	6.24(s)	120.3	7,9,12,13	14
12	–	167.0	–	
13	–	79.3	–	
14	4.25(dd,10.4,5.7)	71.2	12,13,15,16	11,16,26
15ax 15eq	2.04(ddd,14.2,12.4,10.7) 2.00(m)	31.9	3,13,14,16 3,13,14,16,17	25
16	2.84(m)	45.2	3,4,14,15,17,25	14,26
17eq 17ax	2.71(dd,13.2,6.4) 2.45(dd,13.2,10.6)	27.2	3,16,18 15,16,18	 25
18	–	117.3	–	
19	–	125.0	–	
20	7.41(br d, 6.9)	118.5	18,19,22,24	17eq
21	7.06(ddd, 7.0, 7.0, 1.6)	119.7	19,23	
22	7.08(ddd,7.0, 7.0, 1.6)	120.7	20,24	
23	7.27(br dd, 7.0, 1.6)	111.5	19,21,24	1
24	–	139.8	–	
25	1.36(s)	16.2	2,3,4,16	5ax,15,17ax
26	1.21(s)	23.1	3,4,5,13	5eq,6ax,14,16
27	–	78.4	–	
28	1.42(s)	28.8	9,27,29	9,29
29	1.17	23.2	9,27,28	9,28

[a]NOESY correlations for scalar-coupled protons are not included.

Table 3. NMR Data For 14-(N,N-Dimethylvalyloxy)paspalinine

Position [a]	^1H	^{13}C	HMBC Correlations
1	7.70 (s)	–	2, 18, 19, 24
2	–	150.7	–
3	–	51.2	–
4	–	41.0	–
5ax 5eq	2.69(m) 1.81(m)	27.7	3, 4, 6, 7, 26 4, 6, 13, 26
6ax 6eq	2.86(m) 1.99(m)	28.6	4, 5, 7 4, 7, 12
7	–	104.6	–
9	4.29(s)	88.4	7, 10, 11, 27, 28
10	–	196.1	–
11	5.60(s)	119.5	7, 9, 12, 13
12	–	166.0	–
13	–	79.1	–
14	5.36(dd,10.5,5.5)	74.6	1', 15
15ax 15eq	2.28(m) 2.03(m)	28.9	3, 13, 14, 16, 17 3, 13, 14, 16
16	2.86(m)	45.1	3, 4, 14, 17, 25
17eq 17ax	2.74(m) 2.45(dd,13.0,10.7)	27.2	2, 3, 16, 18 2, 15, 16, 18
18	–	117.5	–
19	–	125.0	–
20	7.42(br d, 6.8)	118.6	22, 24
21	7.07(m)	119.9	19, 23
22	7.09(m)	120.9	20, 24
23	7.28(br d, 7.0)	111.6	19, 21
24	–	140.0	–
25	1.40(s)	16.4	2, 3, 4, 16
26	1.26(s)	23.2	3, 4, 5, 13
27	–	78.5	
28	1.41(s)	28.6	9, 27, 29
29	1.15(s)	23.1	9, 27, 28
1'	–	169.6	–

2'	2.73(br d, 10.0)	74.4	1', 3', 4', 5', 6', 7'
3'	2.06(m)	27.3	1', 2', 4', 5'
4'	0.96(d, 6.6)	19.1	2', 3', 5'
5'	0.94(d, 6.6)	20.0	4'
6'/7'	2.33 (s)	41.6	2', 6', 7'

*Axial and equatorial assignments are based on comparison with data from 14-Hydroxypaspalinine.

On the basis of HREIMS and ^{13}C NMR data, 14-hydroxypaspalinine was determined to possess the molecular formula $C_{27}H_{31}NO_5$. This formula differed from that of paspalinine by the addition of one oxygen atom. As expected, the NMR data revealed close similarities between the two compounds, specifically indicating that one of the five methylene carbons of paspalinine is replaced by an hydroxylated methine (71.2 ppm) in 14-hydroxypaspalinine. The proton spin systems in 14-hydroxypaspalinine were identified by analysis of a ^1H-^1H COSY spectrum recorded at 600 MHz. Although these data showed that the position of hydroxylation could not be C-17 (the proton signal is a doublet of doublets), they did not unambiguously eliminate the four other possible positions, so further information was required. Shift assignments for carbons bound to hydrogen atoms were established on the basis of HMQC [Bax and Subramanian, *J. Magn. Res.* 67:565 (1986)]. The remaining carbon NMR assignments and the organization of the spin systems were determined with the aid of an HMBC experiment [Bax and Summers, *J. Am. Chem. Soc.* 108:2093 (1986)] as summarized in Table 2. HMBC correlations of the methine proton at 4.25 ppm with signals for carbons 12, 13, 15, and 16 indicated the secondary alcohol group location at position 14. All other HMBC correlations are consistent with the proposed structure, and the remainder of the NMR and mass spectral data for this compound support the assignment of the structure as shown.

The relative stereochemistry at the new chiral center was established through analysis of NOESY data and ^1H NMR *J*-values as shown in Table 2.

In the NOESY experiment, correlations of H-14 with H-16 and H_3-26 were observed. In addition, the signals for H-16 and H_3-26 showed correlations to H-14 and to each other. These data provide evidence for a 1,3,5-triaxial arrangement of protons 14, 16 and the methyl group H_3-26, indicating the relative stereochemistry at position 14 as shown. This stereochemistry is consistent with the *J*-values observed for H-14, which include an axial-axial coupling with H-15_{ax} (10.7 Hz). The relative configurations at the other chiral centers are the same as those of paspalinine, and the remainder of the NOESY data support this assignment.

Proton and carbon NMR spectra for 14-(N,N-dimethylvalyloxy)paspalinine ($C_{34}H_{44}N_2O_6$ based on HRFABMS) contained signals that matched closely with those for 14-hydroxypaspalinine. The only significant differences were a downfield shift of H-14 (from 4.25 to 5.36 ppm), an upfield shift of H-11 (from 6.24 to 5.60 ppm), and the presence of resonances accounting for the additional carbon atoms and protons indicated by the molecular formula. These observations suggested that 14-(N,N-dimethylvalyloxy)paspalinine differed from 14-hydroxypaspalinine by acylation at the 14-OH with a $C_7H_{14}NO$ unit, and were verified by analysis of COSY, HMQC, and HMBC data for 14-(N,N-dimethylvalyloxy) paspalinine. The ^{13}C NMR signals associated with the acyl subunit consisted of a carboxyl carbon, four methyl carbons (two bound to nitrogen), and two methine carbons. ^1H NMR and COSY data demonstrated that the two methine protons are coupled to each other, and that the upfield methine (2.06 ppm) is part of an isopropyl group. HMBC correlations of the downfield methine proton (2.73 ppm) with the carboxyl, N-methyl, and isopropyl group signals, and correlation of the isopropyl methine with the carboxyl carbon signal, indicated that the acyl group is an N,N-dimethylvalyl unit. Petit et al.[*J. Am. Chem. Soc.* 113:6692 (1991)] describe the natural occurrence of the N,N-dimethylvalyl unit as an amino acyl unit in the dolastatins, a family of small peptide antineoplastic agents isolated from sea hares.

The site of connection of the acyl group to the hydroxypaspalinine core structure was determined based on the downfield shift of the H-14 resonance in 14-(N,N- dimethylvalyloxy)paspalinine compared with the non-acylated compound 14-hydroxypaspalinine. This connection was confirmed after analysis of a selective INEPT experiment [Bax, *J. Magn. Res.* 57:34 (1984)], which afforded a 3-bond correlation of the H-14 resonance to the carboxyl carbon signal of the N,N-dimethylvalyl group (169.6 ppm).

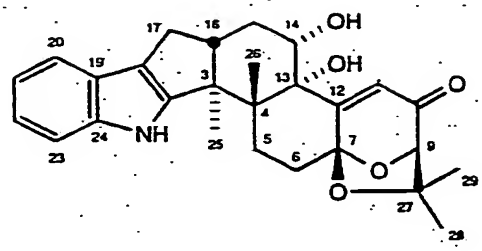
Example 4: Insecticidal Activity of 14-Hydroxypaspalinine and 14-(N,N-Dimethylvalyloxy)paspalinine

The compounds were evaluated by the procedure described in example 2 using a single neonate *H. zea*. 14-Hydroxypaspalinine and 14-(N,N-dimethylvalyloxy) paspalinine cause a 91% and 82% reduction in weight gain respectively relative to controls after one week when incorporated into a standard *H. zea* diet at 100 ppm.

While the invention is susceptible to various modifications and alternative forms, specific embodiments thereof have been shown by way of example and were herein described in detail. It should be understood, however, that it is not intended to limit the invention to the particular forms disclosed, but on the contrary, the intention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the invention as defined by the appended claims.

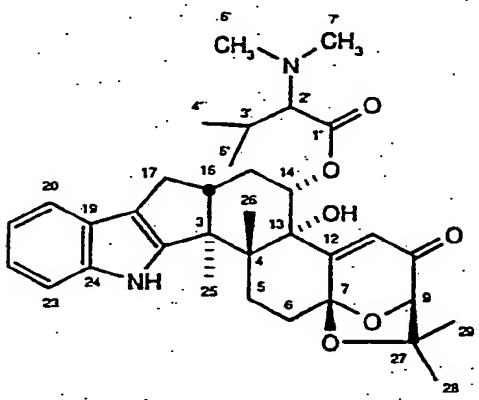
WE CLAIM:

1. A substantially pure indole designated 14-hydroxypaspalinine having the formula:



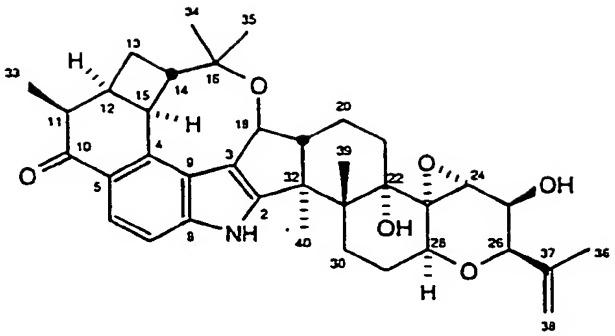
or

14-(N,N-dimethylvalyloxy)paspalinine having the formula:



or

10-oxo-11,33-dihydropenitrem B having the formula:



2. A composition for controlling insects comprising: an insecticide selected from the group consisting of 14-hydroxypaspalinine, 14-(N,N-dimethylvalyloxy)paspalinine, and 10-oxo-11,33-dihydropenitrem B; and an inert carrier.

3. The composition of claim 2 including an amount of the insecticide effecting insects of the Coleoptera species.

4. The composition of claim 2 including an amount of the insecticide effecting *Carpophilus hemipterus*.

5. The composition of claim 2 including an amount of the insecticide effecting insects of the Lepidoptera species.

6. The composition of claim 2 including an amount of the insecticide effecting *Helicoverpa zea*.

7. A method of controlling insects comprising applying an effective amount of an insecticide selected from the group consisting of 14-hydroxypaspalinine, 14-(N,N-dimethylvalyloxy)paspalinine, and 10-oxo-11,33-dihydropenitrem B to a locus of insects.

8. The method of claim 7 wherein the insects are Coleoptera species.

9. The method of claim 7 wherein the insects are *Carpophilus hemipterus*.

10. The method of claim 7 wherein the insects are Lepidoptera species.

11. The method of claim 7 wherein the insects are *Helicoverpa zea*.

INTER TIONAL SEARCH REPORT

PCT/US93/01834

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07D 491/22; A01N 43/90
US CL :548/417; 514/410
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 548/417; 514/410

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A, 5,068,349 (Kanda et al.) 26 November 1991	1-11
A	Trans. Br. mycol. Soc.; (1988); Wicklow, et al.; Vol. 91(3); "Sclerotial Metabolites of Aspergillus Flavus Toxix to a Detritivorous Maize Insect (Carpophilus Hemipterus, Nitidulidae)", pages 433-438; (see Fig. 2, page 437).	1-11
A	Tetrahedron Letters; (1980); Springer et al; Vol 21; "Paspaline and Paspalicine two Indole- Mevalonate Metabolites from Claniceps Paspali" pages 231-234; (see formulas 2 and 3, pages 231).	1-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
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Date of the actual completion of the international search 05 JULY 1993	Date of mailing of the international search report 20
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer Ngoctho Nguyen for JOSEPH PAUL BRUST NGUYEN NGOC-THO INTERNATIONAL DIVISION Telephone No. (703) 308-1235

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. Chem. Soc. Perkins Trons I; (1983); de Jesus, et al.: "Tremorgenic Mycotoxins from Penicillium crustosum: Isolation of Penitrems A-F and the Structurs Slucidation and Absolute Configuration of Penitrem A'", pages 1847-1856; (see formula "(4)", pages 1847).	1-11
A	Titrahedron Letters; (1980); Gallagher et al; Vol. 21, "Paspalinine, A Tremorgenic Metabolite from Claviceps Paspali Stenens et Hall", pages 235-238 (see formula 2, pages 235).	1-11
A	Tetrahedron Letters; (1980); Gallagher et al; Vol. 21, "Aflatrem, A Tremorgenic Toxins from Aspergillus Flavus", pages 239-242 (see formula 2, pages 239).	1-11